(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 November 2001 (15.11.2001)

PCT

(10) International Publication Number
WO 01/85341 A1

(51) International Patent Classification[7]: B01L 3/00, B01J 19/00

(21) International Application Number: PCT/GB01/02119

(22) International Filing Date: 14 May 2001 (14.05.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0001768-1 12 May 2000 (12.05.2000) SE

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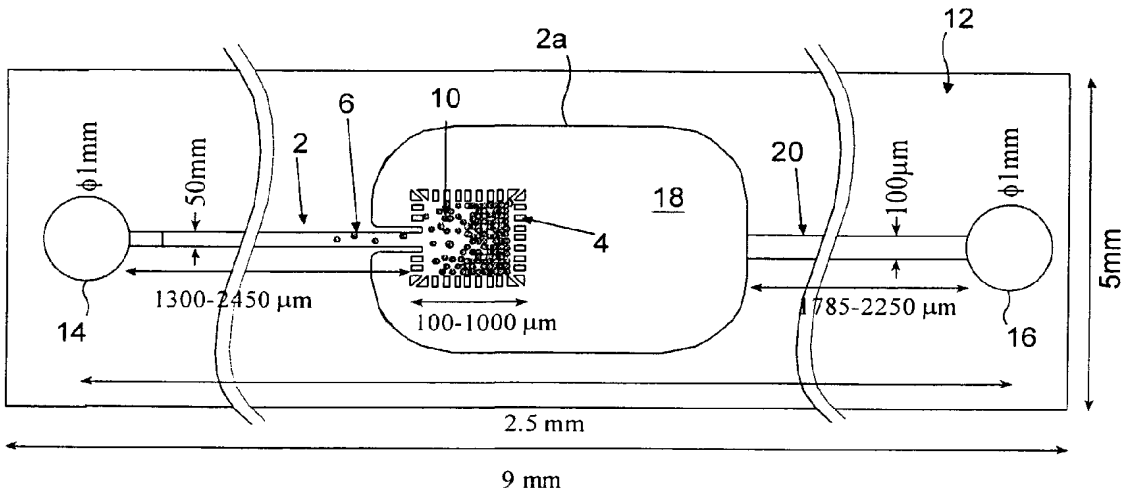
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EC, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MICROFLUIDIC DEVICES



(57) Abstract: A microfluidic device for trapping nonmagnetic and magnetic beads (6) is disclosed which device has an inlet (2), an outlet (20) and a bead trapping filter (4) wherein said bead trapping filter comprises a wall with slots wherein the openings of the slots is less than the diameter of the beads. The filter (4) is provided in an enlarged zone (2a) and may extend around the flow axis - e.g. a box-like shape. The device may be used in a method of sequencing-by-synthesis.

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Microfluidic Devices

The present invention relates to microfluidic devices and particularly, although not exclusively, to microfluidic devices for manipulating microbeads.

Microspheres, also known as beads, are routinely used as the mobile solid phase in medical diagnostics, microbiology, cancer research, immunology and molecular biology for separation, synthesis and detection of molecules. The uniformity of the beads and their precisely defined size ensure that each bead has identical chemical and physical properties. Beads are available in several different materials and sizes (a few nanometers to millimeters in diameter). The surface chemistry of the beads can be modified with various functional groups rendering the beads hydrophobic, hydrophilic, fluorescent, or active towards special ligand binding proteins.

In order to perform and detect chemical reactions on beads, they must be confined to a limited volume. Microfluidic devices for manipulating microspheres have, to date, mainly involved magnetic microspheres and have primarily focused on magnetically activated separations, see for example P. Telleman, U. Larsen, J. Philip, G. Blankenstein, and A. Wolff, Cell sorting in microfluidic systems, *Micro Total Analysis Systems '98, Banff Canada, Oct 13-16, 1998, 39-44.*

Paramagnetic beads, *i.e.* beads with a magnetite (Fe_3O_4) core sealed in a polymer shell, are extensively used today because they can be conveniently separated by applying external magnets. However, magnetic principles are not always advantageous in micro total analysis systems (μ-TAS) applications. External magnetic systems complicate precision handling and result in a bulky system. Incorporation of magnetic components on wafer level is also a very complicated process.

It is an object of the present invention is to provide an improved way of manipulating both nonmagnetic and magnetic beads.

When viewed from a first aspect the invention provides a microfluidic reaction apparatus for trapping one or more particles of predetermined nominal size or range of sizes, comprising a flow inlet and a reaction zone having an enlarged

cross-sectional area in comparison to said flow inlet, said reaction zone comprising a filter means having a plurality of holes defined therein, the holes being smaller than said nominal size or range of sizes and arranged so as to trap said particles while a fluid flows from the flow inlet through the filter means.

By providing a through-flow system in accordance with the invention as opposed to a closed one, such as a microtiter plate, reactions may proceed more efficiently and be sensed more accurately since there will not be an accumulation of by-products as the reaction progresses. Furthermore, it will be seen by those skilled in the art that in accordance with the invention a through-flow passage in a microfluidic reaction apparatus is widened where the filter is placed to trap particles. This means that in comparison with prior art arrangements, a greater number of holes may be provided for a given hole size. This is beneficial in reducing the tendency of the filter to clog and promotes homogenous flow of the liquid over the filter by the liquid. This enhances the feasibility of miniaturising many experiments, assays etc. that are currently performed in test tubes, microtiter plates and the like, utilising the large assortment of beads and the like which are available.

Furthermore, since the reagent fluid can flow through the apparatus without disturbing the particles or their surface functional groups, the apparatus may be used to implement multi-step reactions at a single location.

It should be appreciated by those skilled in the art that the enhanced flow characteristics which may be achieved in accordance with the invention, in particular a reduced tendency to clog, are beneficial in whichever stage of a process it is required to pass fluid over the filter and not necessarily during the reaction itself. Thus whilst some preferred applications of an apparatus in accordance with the invention involve flowing a reaction fluid over the particles in a through-flow process, it is equally possible for the apparatus to be used for 'stop-flow' measurements in which there is not a significant flow of reaction fluid through the filter. In such cases the improved fluid characteristics mentioned above are still beneficial when charging the filter with the particles using a fluid in which the particles are suspended and/or in performing washing steps between the respective stages of a multi-stage reaction.

Although presently preferred applications of the invention are for reactions in which an analyte is immobilised on a bead of some sort, it will be appreciated that

apparatus in accordance with the invention may also be used in applications not involving beads – e.g. cell-cell separations, cell deformability tests and particle filtration [6,7].

The filter means could extend laterally across the flow zone, i.e. normal to the direction of liquid flow. Preferably however, the filter means extends around the flow axis, i.e. it has at least some extent in a plane whose normal is not parallel with the flow axis. This is beneficial since it enhances the concentration of the trapped particles into a smaller space which aids detection of the reaction. This is important, for example, when the reaction is one which emits light since it will increase the intensity and thus ease of measurement of the potentially low level of light. In particularly preferred embodiments the filter means extends substantially completely around the flow axis so as to form a porous reaction chamber. This enables the particles to be trapped in a compact arrangement whilst still allowing good porosity for the fluid passing through. Preferably the chamber is shaped so as to conform to the shape of a reaction monitoring device. Thus in one preferred embodiment for example, the enclosure is substantially rectangular to match the rectangular shape of a charge coupled device. Of course an array of such devices could be used in which case the array may have a different overall shape. Alternatively the charge-coupled device may not be rectangular – e.g. it may be hexagonal in which case the chamber would preferably be hexagonal too. In another possible embodiment the chamber could be circular or partly or substantially spherical.

It will be appreciated that confining the particles to a porous reaction chamber corresponding in shape to a detection device is beneficial in its own right, regardless of flow characteristics. Thus when viewed from a further aspect the invention provides a reaction apparatus comprising a porous reaction chamber for trapping one or more particles therein and a reaction monitoring means arranged to monitor the particles trapped in the reaction chamber; wherein the reaction chamber is arranged so as substantially to correspond in shape to the reaction monitoring means.

The holes may be of any convenient size or shape as long as they are smaller in at least one dimension than the nominal size or range of sizes of the particles. It should be understood that where particles are non-spherical it is the minimum dimension that is taken to represent the 'size' of the particle. The important criterion

is that the particles are trapped by the holes without passing through. Preferably the holes are elongate, most preferably rectangular. This promotes a substantially lateral flow with minimal 'dead spaces' i.e. regions with low or no flow.

The holes could be defined as apertures in a wall or between the elements of a mesh, but preferably are defined between a plurality of preferably substantially parallel discrete wall elements which are preferably rectangular. Such wall elements preferably extend normally from a substrate to form pillars.

Apparatus in accordance with the invention could be open on one side, but are preferably closed to prevent contamination. In preferred embodiments at least the reaction zone of the apparatus is closed by a substantially transparent cover. This allows reactions generating light or modifying incident light, for example fluorescence resulting from shining laser light onto the particles, to be monitored.

The apparatus is preferably formed on substantially planar substrate. Also preferred is that a flow outlet is provided, preferably substantially colinearly with the flow inlet and the reaction zone.

Apparatus in accordance with the invention can be manufactured using standard photolithographic procedures and bulk micromachining of silicon. Preferably a mask fabrication process is used involving a deep reactive ion etching (DRIE). Most preferably a two stage process is used to form respective faces from a substantially planar substrate.

Although a wide range of different micro total analysis systems have been demonstrated [4], efficient standard interconnections between these devices and the macroscopic world are not yet available [5]. It is a further object to provide an improved method for forming such an interconnection and according to a further invention disclosed herein there is provided a method of attaching an external tube to a channel in a micro-reaction apparatus comprising placing a guide member into the channel, sliding said tube along said guide member and bonding an end of said tube onto the mouth of the channel.

The bond is preferably effected by heating the mouth of the channel so as partially to melt the end of the tube. In preferred embodiments the strength of the bond is enhanced by applying an adhesive around the base of the tube. In the

preferred embodiment in which the channel is etched in silicon-glass and the tube is polyethylene, the adhesive is an epoxy glue.

Preferably the mouth of the channel is roughened prior to bonding the tube thereto. This enhances the strength of the bond.

The apparatus of the invention may be used for many different purposes. A preferred use of the apparatus is in the analysis or sequencing of nucleic acid (i.e. DNA, RNA or cDNA). A particularly preferred nucleic acid sequencing application is in sequencing-by-synthesis. Any suitable method of detecting nucleotide incorporation in sequencing by synthesis reactions may be used, i.e. use of fluorescently labelled nucleotides, colourimetric detection, radiolabels, or use of enzymatic detection systems. Most preferably the apparatus is used in the Applicant's PyroSequencing™ sequencing-by-synthesis technique full details of which are given in WO98/13523. It is therefore preferred that the analyte in question comprises a nucleic acid, i.e. DNA fragment and that the liquid comprises a nucleotide. Preferably the nucleic acid fragment is single-stranded. Apparatus in accordance with the invention are particularly beneficial in such techniques since they allow different nucleotides to be cyclically pumped over the particles, preferably microbeads such as Dynabeads™ (available from Dynal Biotech ASA, Norway), which remain in the same position and thus are easy to monitor for the generation of light associated with the technique.

In the context of this application, the term nucleotide will be understood to cover both deoxy and dideoxy nucleotide triphosphates (dNTPs and ddNTPs). Further, analogues of dNTPs and ddNTPs which are normally incorporated into a growing DNA chain by a polymerase are also included.

Indeed the use of a through-flow system for such techniques is novel and inventive in its own right and thus when viewed from a further aspect the invention provides a method of sequencing by synthesis comprising trapping a target immobilised on a plurality of beads, on a filter means provided in a through-flow channel and passing at least one nucleotide over said trapped beads.

Certain preferred embodiments of the invention will now be described, by way of example only, with reference to the accompanying drawings in which:

Figure 1a shows a prior art filter arrangement;

Figures 1b and 1c show different embodiments of filter arrangements in accordance with the present invention;

Figure 2 shows schematically a plan view of an embodiment of an apparatus in accordance with the present invention using the filter arrangement of Fig. 1c;

Figure 3 shows a scanning electron microscope (SEM) view of an embodiment of a device in accordance with the present invention;

Figures 4a and 4b show SEM images of an embodiment of a reaction chamber in accordance with the present invention;

Figure 5 shows a SEM view of pillars in the reaction chamber of figure 5;

Figures 6a-6d show respective stages in a method in accordance with an invention disclosed herein for attaching fluid connectors;

Figure 7 illustrates the principle of allele specific pyro-extension on a SNP;

Figure 8 shows a plot of total collected light versus time; and

Figures 9a and 9b show snapshots of respectively match and mis-match pyro-extension.

Turning to Figs. 1a to 1c, three different schematic filter and channel arrangements may be seen. Fig. 1a shows a known filter arrangement in which a planar filter A is placed laterally across a uniform channel B. This arrangement is prone to clogging when packed with beads C, thus impeding the free flow of liquid through the channel.

Fig. 1b shows a filter arrangement in accordance with the invention. In this embodiment the fluid inlet channel 2 opens into a zone 2a of enlarged cross section, compared to the inlet channel, in which a filter 4' is disposed laterally across it. For a given size or range of sizes of particles 6, and hence hole size in the filter 4', a greater number of holes is provided than the arrangement of Fig. 1a. This significantly reduces the tendency for the filter 4' to clog in comparison with Fig. 1a, and results in more homogeneous flow characteristics over it.

The arrangement in Fig. 1b is an improvement over that in Fig. 1a from the point of view of fluid flow characteristics. However in some circumstances it may be disadvantageous for the beads 6 to be more spread out than in the Fig. 1a arrangement, e.g. where the reaction which takes place on surface of the beads generates a low level of light.

The arrangement shown in Fig. 1c overcomes this problem. In this arrangement the filter 4 is similarly disposed in a zone 2a of enlarged cross-section. However in this embodiment, rather than being planar, the filter 4 extends all the way around the flow axis 8 to form a porous reaction chamber 10. This confines the beads 6 in a more compact region within the chamber 10, thereby enhancing the intensity of light emitted for example, whilst giving an increased number of holes which promotes further improved fluid flow in comparison to the arrangements of Figs. 1a and 1b.

A more detailed plan view of the preferred embodiment is shown in Fig. 2. The microfluidic reaction apparatus is described in a substantially planar, rectangular substrate 12. The method of fabrication is described in greater detail hereinafter. Fluid and particles are introduced to the apparatus by means of a vertically disposed inlet tube 14, extending normally from the other surface of the substrate 12. A similar exit tube 16 is disposed at the other end of the apparatus. The inlet tube 14 is in fluid communication with the inlet channel 2. The inlet channel 2 opens out into a reaction zone 2a of enlarged cross-section. The filter 4 extends all the way around the flow axis 8 to form a box-like reaction chamber 10. The remainder of the enlarged zone 2a forms a waste chamber 18 for collecting fluid that has passed through the filter 4. The waste fluid is channeled into an outlet channel 16 of similar dimensions to the inlet channel 2 and which is in fluid communication with the exit tube 16.

Referring to Figs. 3, 4a, 4b and 5 there may be seen various scanning electron microscope (SEM) images of the embodiment depicted in Fig. 2. From these it may be seen that the filter 4 is made up of a series of parallel vertical pillars 22 extending vertically from the floor of the enlarged zone 2a etched out of the substrate 12. The gaps between the pillars 22 define the holes which must be smaller than the beads or other particles which it is desired to trap.

The apparatus shown in Figs. 2 to 5 is fabricated as follows. First, one hundred millimetre diameter 525 μm thick p-doped silicon (100 off)-wafers are used as the starting material. Photoresist (1.5 μm thick) is used for two etch masks. First, the front side is patterned and etched with the first mask using deep reactive ion etching (DRIE) (Surface Technology Systems, UK) to define the inlet channel 2, reaction chamber 10, filter 4, waste chamber 18 and outlet channel 20. Gas switching in the DRIE process gives rise to the undulating surface pattern on the pillars 22

which may be observed in Fig. 5. To seal the device, a 170, 300 or 500 μm thick Pyrex glass wafer is anodically bonded to the front side. The backside is then patterned in a second DRIE step with the second mask to create fluid connectors for the inlet and outlet tubes 14, 16. The silicon-glass stack is then sawn into 9x5 mm chips.

External polyethylene (PE) tubes are used as the inlet and outlet tubes 14, 16 and are fixed to the chip in a multi-step procedure as schematically shown in Figs. 6a to 6d. A mask-defined silicon surface roughening is performed around the fluid openings 26 by the second DRIE step to ensure good adhesion of the PE tubes (Fig. 6a). A guide wire 24 is used to align the PE tubes 14, 16 with the respective fluid openings on the chip during the tube fixing process (Fig. 5b). The silicon-glass stack 28 is briefly heated to generate a local melting of the PE tube 14, 16 onto the chip (Fig. 6c). To give additional strength to the assembly, the interface between the chip and the PE tubes 14, 16 is then covered with epoxy glue.

Several exemplary apparatus of the form shown in Fig. 2 were fabricated with varying sizes. The dimensions of these are shown in rows 1 to 8 of Table 1 below. Row 9 relates to a design similar to that in Fig. 1a and is included for the purposes of comparison.

Design	Reaction chamber V (nL)	Pillar W, L, H (μm)	Pillar spacing (μm)	Number of pillars	Inlet Channel W, L, H (μm)	Outlet Channel W, L, H (μm)
1	0,5	3, 10, 50	2	70	50, 2450, 50	100, 2250, 50
2	2,0	3, 10, 50	2	152	50, 2450, 50	100, 2000, 50
3	12,5	3, 10, 50	2	392	50, 2250, 50	100, 1785, 50
4	50,0	3, 10, 50	2	792	50, 1300, 50	100, 1480, 50
5	0,5	3, 5, 50	2	70	50, 2450, 50	100, 2250, 50
6	0,5	3, 20, 50	2	70	50, 2450, 50	100, 2250, 50
7	12,5	3, 10, 50	3	320	50, 1300, 50	100, 1785, 50
8	12,5	5, 10, 50	4	220	50, 1300, 50	100, 1785, 50
9	10	3, 10,50	2	20	50, 2285, 50	100, 2225, 50

Table 1. A summary of the different designs of the flow-through micromachined device where *V* is the filter chamber volume, *W* the pillar width, *L* the pillar length, and *H* the pillar height.

The dimensions of the micromachined structures were measured using a scanning electron microscope and compared with the original specifications. The consistency of the filter pillar dimensions within a reaction chamber and between different reaction chambers was measured.

The dimensions of the measured structures were found to be in good agreement with the original specifications. The micromachined structures were found to have high uniformity indicating a uniform and reproducible fabrication process.

The melt-on method for fixing the external PE tubes to the chip was found to be very convenient and reliable. It resulted in robust, precisely positioned interconnections to the macroscopic world with low dead volumes. It is believed that the epoxy glue is important in giving the assembly the robustness.

The fluid behaviour of the flow-through micromachined device was investigated. To test the ability of the reaction chamber to collect beads and concurrently enable unimpeded flow-through, streptavidin coated beads of two different materials and sizes were used, *i.e.* polystyrene beads with a diameter of 5.50 μm (Bangs Laboratories, IN, USA) and magnetic Dynabeads with a diameter of 2.8 μm (Dynal Biotech ASA, Norway). The bead solutions were applied manually with a pipette under a standard light microscope with 40X objective. To enable detailed observations of individual beads in the microfluidic device, the beads were applied at

a low concentration (10 000 beads/mL). Samples at the outlet were collected and controlled under the microscope to confirm that beads do not pass through the filter.

To investigate the fluid behaviour of the microfluidic device on its own, water without beads was first applied. The flow rate for water was about 3.5 μL/min for all the designs presented in table 1 when a constant pressure of 3.0 kPa was applied at the inlet. The flow rate was determined by measuring the speed of the liquid column. The different filter dimensions and number of pillars proved to have no significant influence on the pressure drop for water.

Next the respective reaction chambers were packed with beads. It was observed through the microscope that the chambers were easy to pack. The beads passed freely through the inlet channel and packed the reaction chamber from bottom to top. All designs successfully capture beads with a diameter of 5.50 μm. No beads were observed to pass through the filter, hence no beads were found in the filtered liquid at the outlet when examined under the microscope.

The smallest reaction chamber (design 1, 5, 6) has a volume of 0.5 nL and can hold about 50 beads with a diameter of 5.50 μm. There is no upper limitation of the flow-through volume of liquid or gas, which is important when working with very low sample concentrations. The smallest volume required to fill the device is 3.0 nL (the volume of the inlet channel and reaction chamber).

This time, the flow rate for water was found to be about 2.2 μL/min (again when a constant pressure of 3.0 kPa was applied at the inlet) for each of designs 1-8 when the reaction chamber is completely packed with beads. Hence, the flow rate decreases by about 40% when the reaction chamber is packed with beads. The different dimensions of the reaction chamber and number of pillars did not affect the flow rate significantly. The results are shown in Table 2.

Design	Calculated pressure drop across the inlet (kPa)	Calculated pressure drop across the outlet (kPa)	Calculated pressure drop across the filter (kPa)	Calculated total pressure drop (inlet, outlet and filter) (kPa)	Applied pressure (kPa) to obtain a flow rate of 3.5 µL/min	Calculated vs. applied pressure drop (%)
1	1.7	0.5	0.3	2.5	3	83
2	1.7	0.4	0.1	2.2	3	73
3	1.6	0.4	0.04	2.0	3	67
4	0.9	0.3	0.02	1.2	3	40
5	1.7	0.5	0.1	2.3	3	77
6	1.7	0.5	0.5	2.7	3	90
7	1.6	0.4	0.02	2.0	3	67
8	1.6	0.4	0.01	2.0	3	67
9	1.6	0.5	0.9	3.0	3	100

Table 2. Calculated and applied pressure drop (at the inlet) for a constant flow rate of 3.5 μL/min.

Analytical calculations were performed to verify the pressure drop over the different components, *i.e* the inlet and outlet channel, and the filter in the reaction chamber without beads. Poiseuille's law was used for calculating the pressure drop over the channels [8]. The pressure drop over the filter was calculated as for a number of parallel short channels using

$\Delta P = Q_v C \mu / 2 A D_h^2$ (Equation 1)

where ΔP is the pressure drop, Q_v the volumetric flow, C the friction coefficient (96 for rectangular cross section w>>h), μ the fluid dynamic viscosity, A the cross sectional area of flow path and D_h the hydraulic diameter [9]. Equation 1 is valid when $2<L/D_h<50$ where L is the length of the channel [8]. In table 2 the calculated pressure drop for the in- and outlet channel and the filter at a flow rate of 3.5 μL/min is presented. For comparison the applied pressure to achieve the same flow rate (3.5 μL/min) is included in table 2. The theoretic and experimental results are in very good agreement. It can be seen that the main pressure drop is across the inlet and outlet channels even for the smallest filter design.

Clogging of the filters is rare and can easily be removed by applying back-pressure. It was concluded that designs 1-8 are much less sensitive to clogging than design 9. This is probably due to the larger filter area present in designs 1-8. Design 9 is a channel with only 20 pillars constituting the filter compared to 70-790 pillars for design 1-8.

Selectivity tests were performed on design 7 and 8 using a mixture of 2.8 μm and 5.50 μm beads. The larger beads were efficiently captured in the reaction chamber while the smaller beads easily passed through the filter.

Gas bubbles present in the samples did not affect the device performance. The beads can easily be removed out of the reaction chamber by applying back-pressure. After removing the beads and carefully cleaning of the micromachined flow-through device, it can be reused.

The flow-through micromachined reaction chamber presented here collects both nonmagnetic and magnetic beads. Nonmagnetic beads have lower density resulting in improved fluid dynamic behaviour in μ-TAS compared to magnetic beads.

The batch fabrication process of the flow-through microfluidic device is simple and reproducible, involving only two masks and two different processing techniques. These are important factors in terms of parallellization and producing cost effective economical μ-TAS. The chip dimensions (9x5 mm) were chosen to simplify practical handling and can be further reduced if required. The smallest reaction chamber of 0.5 nL, collecting approximate 50 beads, can also be further miniaturized if a reduced number of beads or flow-through volume are of interest.

To seal the device, different thicknesses (170-500 μm) of Pyrex were used to enable real time optical detection of the chemical reactions on the beads in the reaction chamber. The thinnest Pyrex wafer will be used for detection of chemical reaction generating only a few photons. Since the sample flow-through does not displace the beads or their surface functional groups multi-step reactions can be implemented at one location in the microfabricated device, facilitating optical detection.

In a preferred application of the present invention, an apparatus as shown in Fig. 2 was used in the Applicant's Pyrosequencing™ sequencing-by-synthesis technique. This technique is performed by hybridizing a sequencing primer to the single-stranded nucleic acid template and incubation with the enzymes DNA polymerase, ATP sulfurylase and luciferase. A specific deoxynucleotide triphosphate (dNTP, nucleotide) is added to the reaction. DNA polymerase catalyses the

incorporation of the nucleotide into the sequencing primer DNA strand, only if it is complementary to the base in the template strand. This technique relies on the detection of pyrophosphate (PPi) which is released upon nucleotide incorporation. The released PPi is converted to ATP by ATP sulfurylase and a proportional amount of detectable light is generated by luciferase. One of the application fields for this technique is analysis of single nucleotide polymorphisms (SNP). In this study two primers with different 3'-ends were used for direct analysis of the variable position. One SNP position in the p53 tumor suppressor gene (exon4, codon72) was selected for analysis. An outer PCR was performed to amplify the SNP site. The outer PCR was followed by an inner PCR generating ~80 bp fragments. One of the inner primers was biotinylated at the 5'-end to allow immobilization. Biotinylated inner PCR product was immobilized onto the streptavidin coated beads. Single-stranded DNA was obtained by incubating the immobilized PCR product in NaOH. The immobilized strand was resuspended in H_2O and annealing buffer was added to the single-stranded templates. The solution was then divided into two wells and primers were added in each well. The primers vary in their 3'-ends and have the sequence 5'-GCTGCTGGTGCAGGGGCCACGG-3' and 5'-GCTGCTGGTGCAGGGGCCACGC-3'. Hybridization was performed and the single-stranded DNA with annealed primer (the substrate) was captured in the reaction chamber. A reagent mixture was then added. The microfluidic device was then placed on a CCD camera and the collected data was analyzed.

The principle of allele specific pyro-extension on the SNP used in this study is illustrated in Figure 7. If the 3'-end of the primer matches to the DNA template (a) and a reagent mixture containing all four nucleotides is added to the annealed template, a light signal will be produced. This signal indicates that the DNA polymerase has used the nucleotides to extend the primer and that the released PPi has been converted to ATP and then to light. If the 3'-end of the primer does not match to the DNA template (b), the DNA polymerase will not be able to extend the primer and no light will be produced. This principle was used to analyze the SNP at codon 72 in the p53 gene. The coding SNP at codon 72 involves either a G or C residue corresponding to amino acids proline (CCC) or arginine (CGC). These two variants were analyzed several independent times in a device with a 12.5 nL reaction chamber.

In figure 8, the total amount of collected light is plotted versus time for allele specific pyro-extension. Extension of the match primer resulted in 5 times more light compared to the mismatch extension. A snapshot of the reactions is showed in figure 9, where (a) corresponds to the match and (b) to the mismatch extension in figure 8. Light is detected in the filter-chamber and outlet for the match extension. A weak signal (at the background level) is detected in the filter-chamber for the mismatch extension. The results presented here clearly demonstrate that the SNP analysis can be performed in the micromachined flow-through filter-chamber.

In view of the foregoing description of a preferred embodiment, it will be further appreciated that the sample flow-through rate is adjustable, which is important when performing chemical reactions on beads [10]. Effectively unlimited flow-through volumes of gas and liquid are possible allowing detection of rare molecules or biological species (at or below 100 copies/mL) [11]. The flow-through microfluidic reaction chamber reduces the accumulation of by-products resulting in increased reaction and detection sensitivity compared to a closed system (*i.e.* microtiter plates).

The different dimensions of the reaction chamber of designs 1-8 included in the study do not significantly affect the device performance (*i.e.*, bead capture, air bubble sensitivity, pressure drop) for bead assays as long as the pillars constitute a mechanical barrier. Analytical calculations showed that the largest pressure drop is located across the inlet and outlet channels. The reaction chamber and filter dimensions can therefore be optimized for bead size and chemical reaction parameters. For cell based assays the filter dimensions are important. For example, when filtering cells it is important that the cells pass through the filter as quickly as possible to reduce cell activation, stiction and cell rupture [12].

For performing chemical reactions on the beads in the microfluidic device it is important that the flow resistance remains low when the reaction chamber is packed with beads. Otherwise, it is difficult to pump the reactants through the reaction chamber. For the devices presented here, the flow rate decreases with 40% when the reaction chamber is packed with beads. This corresponds to a flow of about 2 μL/min, which still is well within the margins for μ-TAS.

At least preferred embodiments of the present invention can be used for solid-phase DNA sequencing, automatic introduction of beads by using micropumps, parallellization and device fabrication using plastic replication techniques.

While the filter has been described as being made up of discrete pillars it is also conceivable to form a filter of walls which have slits which have openings which are narrower than the diameters of the beads being filtered. It is also possible to have one or several laterally arranged slits along the perimeter of the reaction chamber. These lateral slits should be narrower than the diameters of the beads being filtered. The pillar filter design creates vertical filter openings which provides a substantially lateral flow with minimal "dead volumes" in the fluidic device (i.e. no or small volumes with low or zero flow). This is a very attractive feature since it improves the quality of the chemical and biochemical analysis. Furthermore, the beads in the reaction chamber are collected in a "point" configuration (e.g. in a circular or quadratic confinement) in order to have the best conditions for detection of the chemical reactions (e.g. using optical detection). The wish for lowest possible flow resistance and a high degree of "point" bead collection configuration leads to circular or quadratic reaction chamber design where as much as possible of the perimeter in the plane is made of filter walls.

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Claims:

1. A microfluidic reaction apparatus for trapping one or more particles of predetermined nominal size or range of sizes, comprising a flow inlet and a reaction zone having an enlarged cross-sectional area in comparison to said flow inlet, said reaction zone comprising a filter means having a plurality of holes defined therein, the holes being smaller than said nominal size or range of sizes and arranged so as to trap said particles while a fluid flows from the flow inlet through the filter means.

2. An apparatus as claimed in claim 1 wherein said filter means extends laterally across said reaction zone.

3. An apparatus as claimed in claim 1 wherein said flow inlet defines a flow axis and said filter means extends around the flow axis.

4. An apparatus as claimed in claim 3 wherein said filter means extends substantially completely around the flow axis so as to form a porous reaction chamber.

5. An apparatus as claimed in claim 4, wherein said porous reaction chamber is substantially rectangular.

6. An apparatus as claimed in claim 4, wherein said porous reaction chamber is circular or partly or substantially spherical.

7. An apparatus as claimed in any preceding claim wherein said holes are defined between a plurality of discrete wall elements.

8. An apparatus as claimed in claim 7 wherein said wall elements are substantially parallel.

9. An apparatus as claimed in any preceding claim wherein said reaction zone is covered by a transparent cover.

10. An apparatus as claimed in any preceding claim comprising a substantially planar substrate defining the flow inlet and reaction zone.

11. A method of reacting a fluid with an analyte immobilised on a plurality of particles, comprising trapping said particles on the filter means of microfluidic reaction apparatus as claimed in any preceding claim and passing said fluid onto the trapped particles.

12. A method as claimed in claim 11 wherein said particles comprise microbeads.

13. A method as claimed in claim 11 or 12 comprising observing the trapped particles for a visible result of said reaction.

14. A method as claimed in claim 13 comprising using a charge-coupled device for detecting light coming from said reaction.

15. A method as claimed in any of claims 11 to 14 wherein said analyte comprises single-stranded DNA immobilised on microbeads.

16. A method as claimed in any of claims 11 to 15 of sequencing by synthesis.

17. A method as claimed in claim 15 or 16 wherein said fluid comprises a nucleotide.

18. A method as claimed in claim 11 wherein said particles comprise biological cells.

19. A method as claimed in any of claims 11 to 18 comprising flowing said fluid over said particles in a through-flow process.

20. A method as claimed in any of claims 11 to 18 comprising flowing said liquid onto the particles trapped by said filter means substantially without the liquid flowing through the filter means.

21. A method of attaching an external tube to a channel in a micro-reaction apparatus comprising placing a guide member into the channel, sliding said tube along said guide member and bonding an end of said tube onto the mouth of said channel.

22. A method as claimed in claim 21 comprising heating said mouth of the channel to cause the end of the tube partially to melt in order to effect said bonding.

23. A method as claimed in claim 21 further comprising applying an adhesive around the base of said tube.

24. A method as claimed in claim 21 comprising roughening the mouth of said channel prior to attaching said tube.

25. Microfluidic device for trapping nonmagnetic and magnetic beads characterised in that it has an inlet, an outlet and a bead trapping filter wherein said bead trapping filter comprises a wall with a plurality of slots with openings having a width which is less than the diameter of the beads.

26. Microfluidic device in accordance with claim 25 characterised in that said wall comprises a plurality of spaced-apart pillars wherein the distance between adjacent pillars is less than the diameter of the beads.

27. Microfluidic device in accordance with claim 25 or 26 characterised in that said pillars are arranged to form a reaction chamber and that all flow from said inlet to said outlet has to pass through said reaction chamber.

28. Microfluidic device in accordance with any of claim 25, 26 or 27 characterised in that said wall is circular or square.

29. A reaction apparatus comprising a porous reaction chamber for trapping one or more particles therein and a reaction monitoring means arranged to monitor the particles trapped in the reaction chamber; wherein the reaction chamber is arranged so as substantially to correspond in shape to the reaction monitoring means.

FIG. 1A

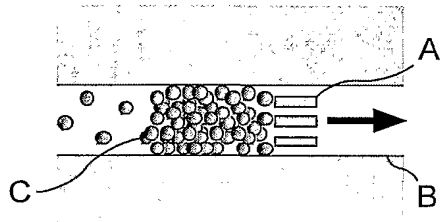


FIG. 1B

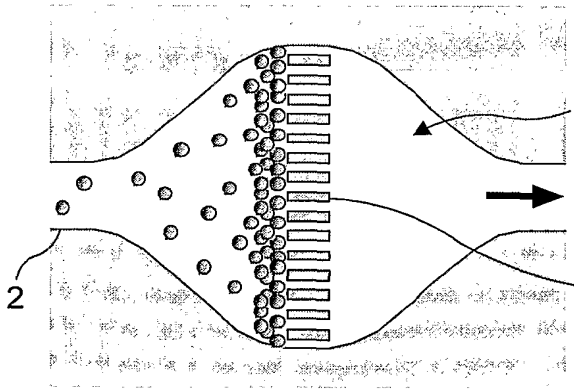
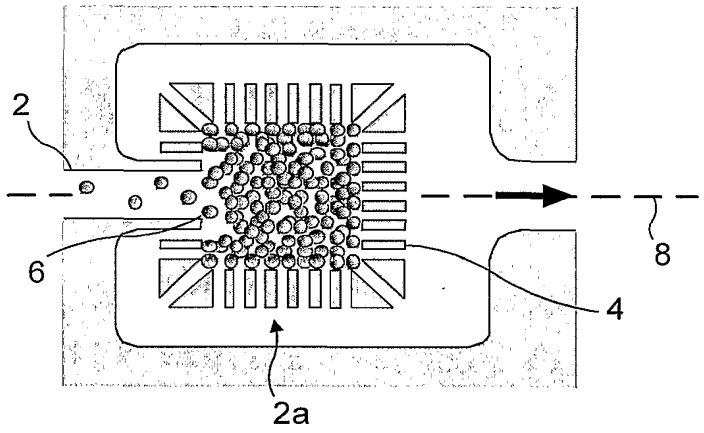


FIG. 1C



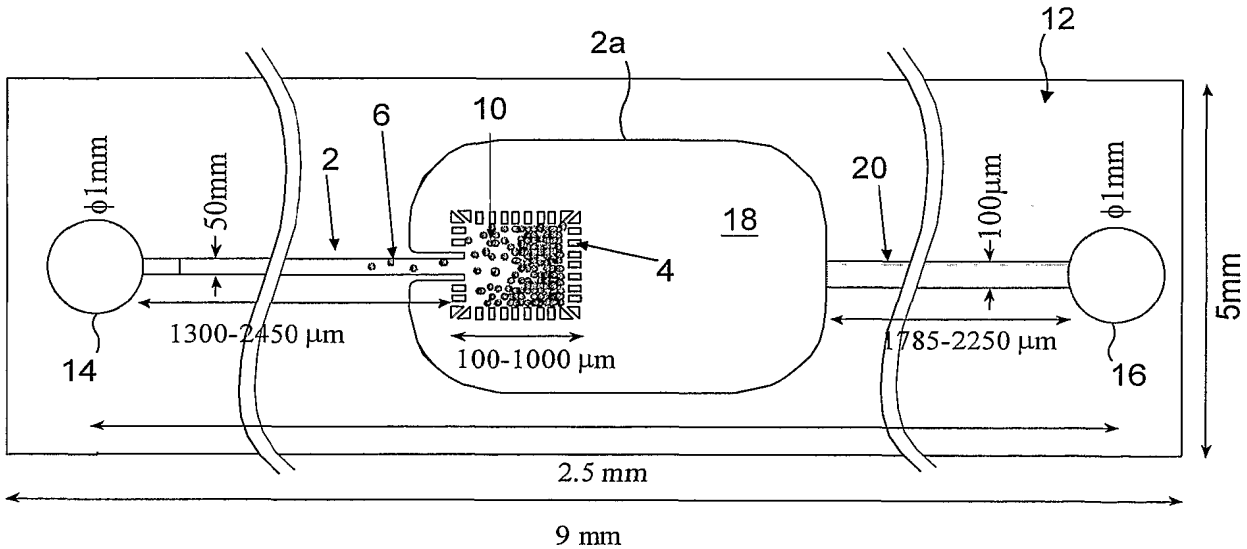


FIG. 2

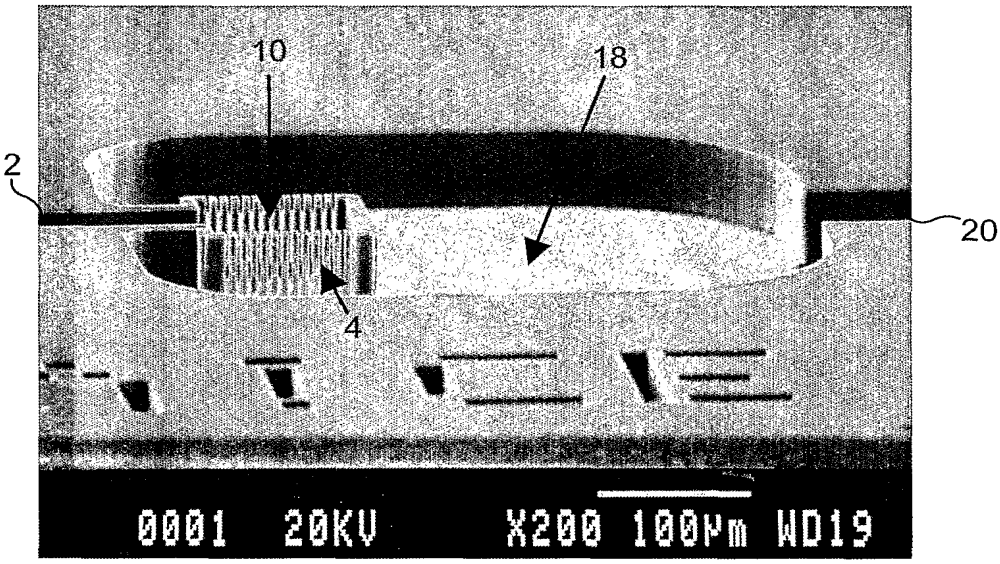


FIG. 3

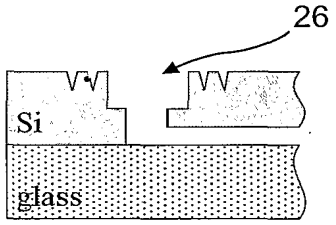


FIG. 6A

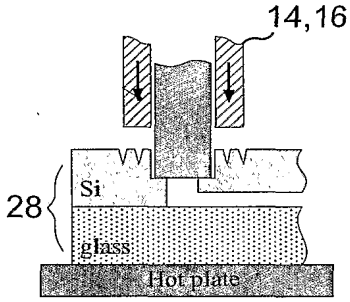


FIG. 6B

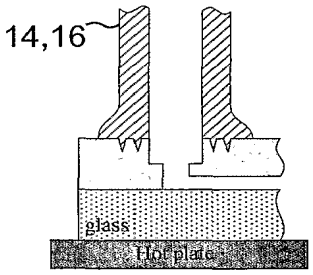


FIG. 6C

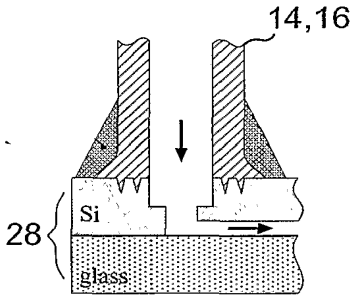


FIG. 6D

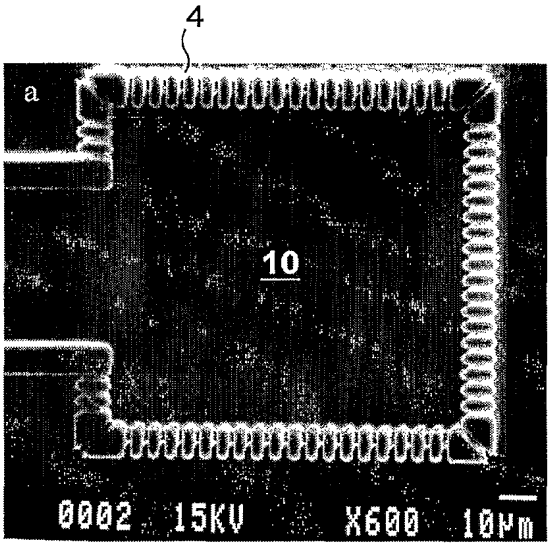


FIG. 4A

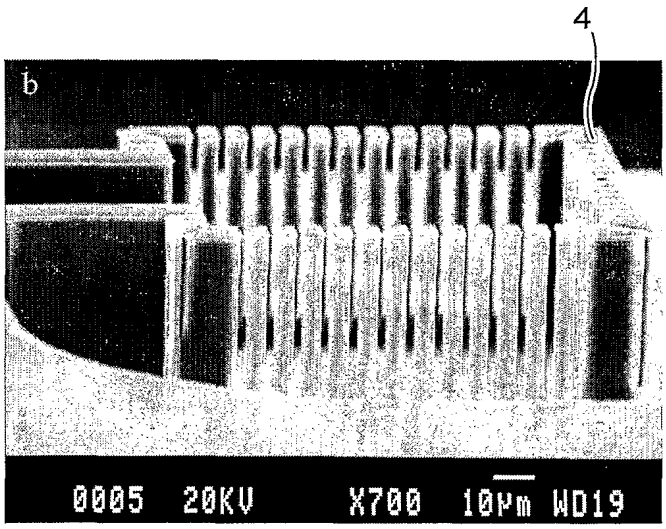


FIG. 4B

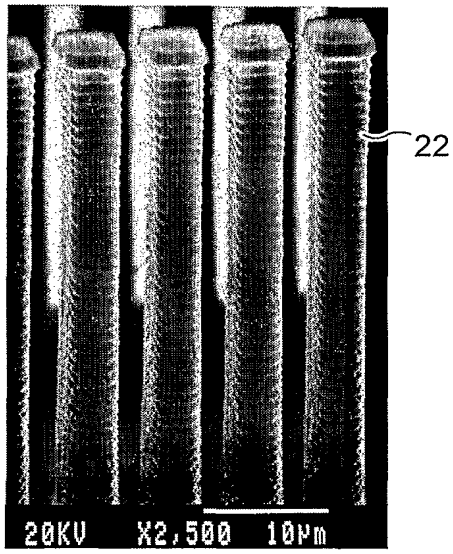


FIG. 5

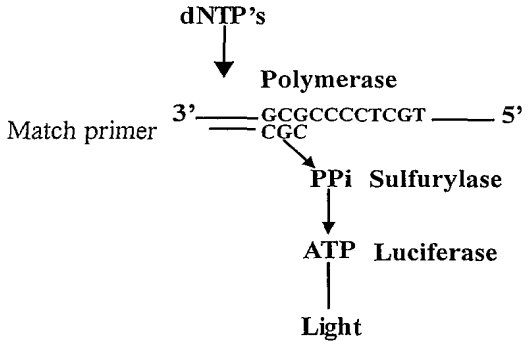


FIG. 7A

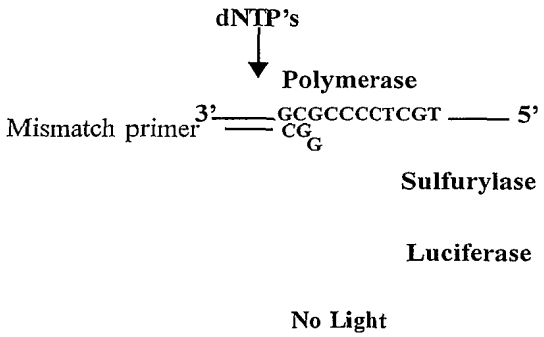


FIG. 7B

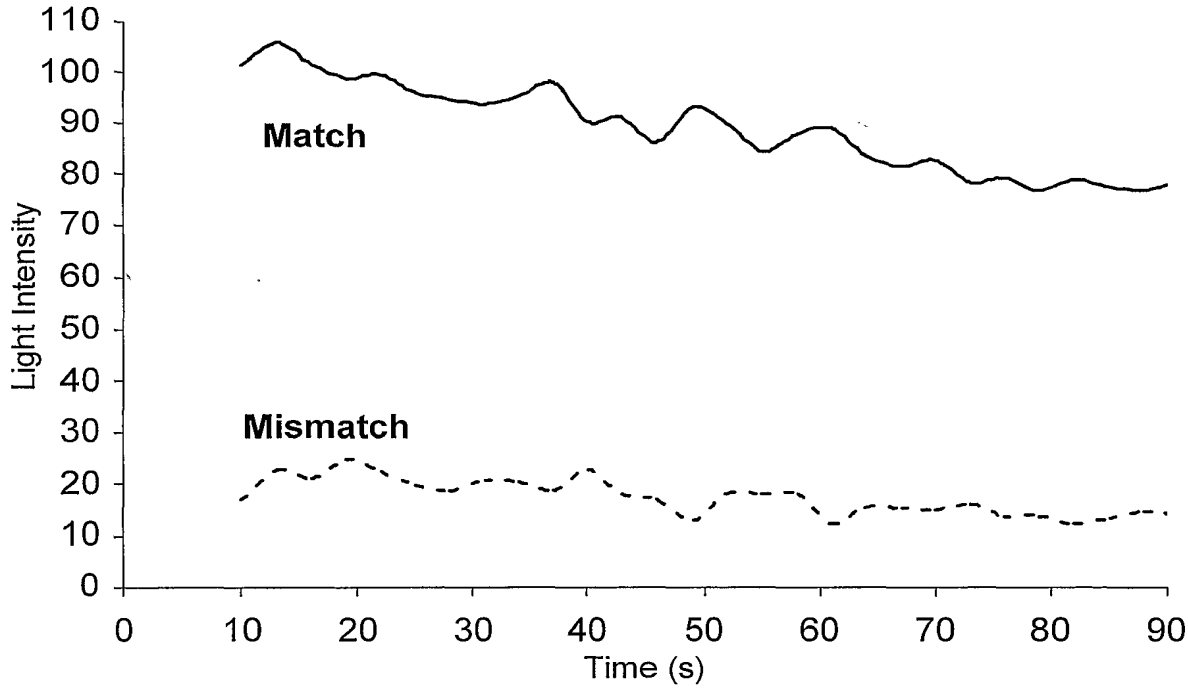


FIG. 8

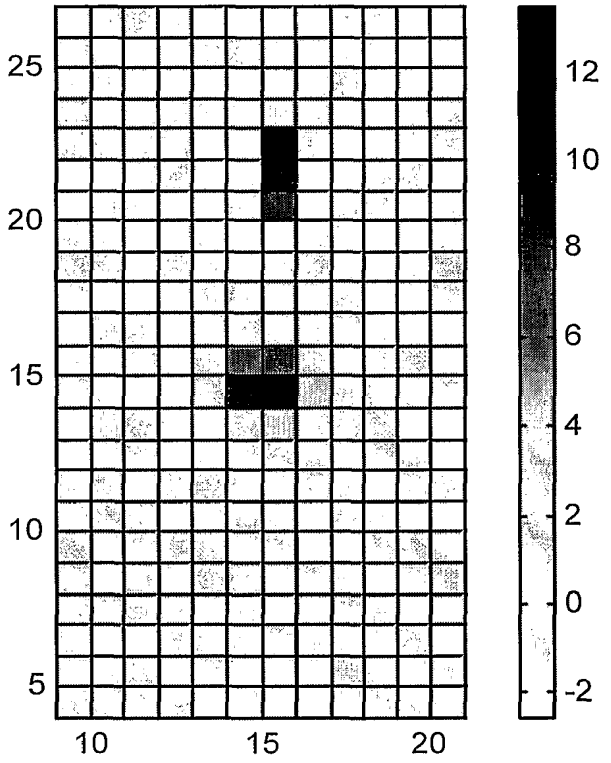

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FIG. 9A

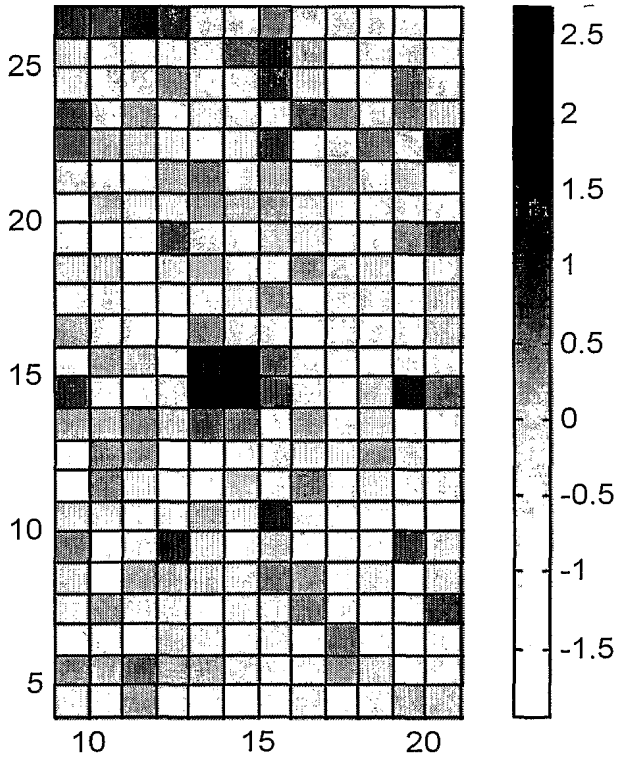
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FIG. 9B

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB 01/02119

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01L3/00 B01J19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01L B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 09042 A (CEPHEID) 25 February 1999 (1999-02-25) abstract; figures 1A,1D,14 page 6, line 20 -page 7, line 19 page 9, line 1 -page 9, line 6 page 19, line 5 -page 19, line 8 page 29, line 1 -page 30, line 27 page 45, line 22 -page 46, line 17	1,2,7,8, 10-12, 15-20, 25-27,29
Y		9,13,14
A		3-6,28

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 July 2001	18. 10. 01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Runser, C

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 01/02119

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 726 026 A (WILDING PETER ET AL) 10 March 1998 (1998-03-10) abstract; figures 1-3 column 5, line 56 -column 5, line 65 column 8, line 48 -column 9, line 17	9,13,14
X A		25,29 1-8, 10-12, 15-20, 26-28

P,X	ANDERSSON H ET AL: "Micromachined flow-through filter-chamber for chemical reactions on beads" SENSORS AND ACTUATORS B,ELSEVIER SEQUOIA S.A., LAUSANNE,CH, vol. 67, no. 1-2, 10 August 2000 (2000-08-10), pages 203-208, XP004213496 ISSN: 0925-4005 the whole document	1-20, 25-29

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 01/02119

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-20,25-29

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-20,25-29

   Microfluidic device for trapping beads

2. Claims: 21-24

   Method for attaching an external tube to a channel in a micro-reaction apparatus

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internation[illegible]plication No
PCT/GL [illegible]1/02119

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9909042 A	25-02-1999	AU 8906698 A	08-03-1999
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